UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 13, 1982

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: Jay Ellenburger, PM12
Insecticide Branch/RD(TS-767)

THRU: R. B. Jaeger, Section Head, Section #1
Toxicology Branch/HED (TS 769)

OKR for RBJ 12/10/82

SUBJECT: Chlorpyrifos-methyl Tox No. 179AA 0,0-dimethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate

Petitioner: Dow Chemical Co.

Action requested:

1. Registration of 464-LLT, Reldan 4E (44.0%) for use on stored grains.

2. Pesticide Petition OF2423
Establish tolerances for the combined residues of chlorpyrifos-methyl and its metabolite 3,5,6-trichloro-2-pyridinol in or on the following commodities.

Grains of barley, corn, oats, rice sorghum and wheat	6.0 ppm
Fat of cattle, goats and sheep	0.2 ppm
Meat of cattle, goats and sheep	0.1 ppm
Meat byproducts of cattle, goats and sheep	1.0 ppm
Fat of hogs and horses	0.3 ppm
Meat of hogs and horses	0.1 ppm

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Meat byproducts of hogs and horses	0.5 ppm
Milk, whole	0.02 ppm
Milk, fat	0.1 ppm
Eggs	0.05 ppm
Poultry meat, fat and meat byproducts	0.05 ppm

Food additive tolerances OH 5277:

Corn oil	160 ppm
Milling fractions (except flour) of corn	20 ppm
Milling fractions of rice	30 ppm
Sorghum Milling Fractions, Except Flour	90 ppm
Soapstock	40 ppm
Oats Milling Fractions, Except Flour	130 ppm
Barley Milling Fractions, Except Flour	90 ppm
Wheat Milling Fractions, Except Flour	30 ppm

Recommendations

1. The registration of 464-LLT should not be granted until the following data are submitted.

   a. The label recommends the application of Reldan 4E for uses that will result in a respirable exposure by the mixer applicator to the spray.

      An acute inhalation study on the formulation is necessary.

   b. The primary eye irritation study is deficient for the lack of the criteria for scoring injury to the eye.

2. The proposed tolerances should not be established until the following studies are submitted.

   a. The acute delayed neurotoxicity study showed chlorpyrifos-methyl to be positive and/or equivocal histopathological evidence of neurotoxicity. In

order to determine the neurotoxic no effect level from repeated exposure, a subchronic neurotoxicity study has been requested (memo of Nov. 12, 1981). The proposed protocol for the subchronic neurotoxicity study should be approved by EPA prior to initiating the study.

b. Mutagenic study identified as Litton Bionetics Report 2547 needs to be repeated according to the current criteria for Ames testing.

c. Teratology study in second species is needed.

Prior Considerations and Deficiencies

1. Registration of 464-LLT
In a review of January 21, 1982, Landolt cited the lack of acute toxicity data to support the registration of Reldan 4E(44%) for use on stored grain.

2. Petition OF2423 and OH5277
In a memo of November 12, 1981, Landolt advised Ellenberger (PM-12) of the status of the review and the then current deficiency in the data profile for this chemical. With the positive and/or equivocal histopathological evidence of neurotoxicity observed in the acute chicken delayed neurotoxicity study, subchronic neurotoxicity study was requested.

Meeting of March 16, 1982 between Dow Chemical with Robert D. Vatne and E.L. Moore, and EPA with R.B. Jaeger, R.E. Landolt and T. Edwards of HED and B. Comfort of RD.

The positive histopathological evidence of delayed neurotoxicity was discussed. It was agreed that we would give consideration to the six month monkey feeding study for its merits in determining the subchronic neurotoxicity from repeated exposure to chlorpyrifos-methyl.

In a review of March 25, 1982 this reviewer made the following recommendations which were contingent to establishing these tolerances.

a. Subchronic neurotoxicity study (Refer to memo of 11/12/81 from Landolt to Ellenburger). The acute delayed neurotoxicity study showed chlorpyrifos-methyl to be positive and/or equivocal histopathological evidence of neurotoxicity. In order to determine the neurotoxic no effect level from

repeated exposure a subchronic neurotoxicity study is required. The proposed protocol for the subchronic neurotoxicity study should be approved by EPA prior to initiating the study.

b. Mutagenic study - refer to memo of 12/21/81 from Mauer to Landolt.

c. Teratology study in second species. In the rabbit teratology study the experimental method is not clear on whether the test material was administered in the diet or by oral intubation.

d. Rat reproduction study. A no observable effect level was not determined in the rat reproduction study (Dow Report NBX-021).

Following the meeting of March 16, 1982 with Dow Chemical Co., T. Edwards of the Toxicology Branch in his secondary review in mid May of the six month monkey feeding study reported on the positive results in the acute chicken delayed neurotoxicity study and observed that the monkey study was "designed to observe inhibition of cholinesterase activity not to observe damage to the nervous systems." For the monkey study the fixing of tissue was not best, perfusion, which was not used, is strongly recommended for all subchronic neurotoxicity studies. Staining of tissue was not adequate for observation of the nervous system. Axon-specific and myelin-specific stain are needed."

Current Considerations

This proposal for tolerances was accompanied by a response from Dow Chemical dated September 14, 1982 to those deficiencies raised in the January 21, and March 25th review of Landolt. The following deficiencies cited in these two reviews by Landolt are followed by a response from Dow Chemical in their letter of September 14, 1982. The response from Dow Chemical is in turn followed by our comment or conclusions.

1. Registration of 464-LLT.
No acute toxicity studies have been received in support of the registration of Reldan 4E 44% chlorpysifos-methyl.

Received from Dow Chemical September 14, 1982, Appendix E

Acute Rat Oral Toxicity
Dow Chemical Co. HET-M-4258(2), April 25, 1980, Acc. 071094

A. Procedure

Four groups of six Fischer 344 male and six female albino rats weighing 85-110 g and 64-95 g respectively, were

fasted overnight and dosed orally at 320, 630, 1300 or 2500 mg/k with the undiluted test material. The animals were observed periodically, weighed weekly and necropsied after 14 days observation.

B. Results

1. Rat oral, male LD_{50} 1803 mg/kg (1258-3833). Slope: N.C. female LD_{50} 1530 mg/kg (1159-2224). Slope: 5:85.

2. Pharmacotoxic signs: At the 1300 mg/kg level, lethargy, ataxia, diarrhea, salivation, tremors and decreased food consumption were observed. Deaths occurred within 2 to 4 day of dosing.

3. Necropsy: One male at the 630 and 1300 mg/kg level exhibited a decrease in thymus size that was related to a decrease in body weight gain.

C. Conclusions

1. Classification of Data - Core Guideline

2. Toxicity Category - III

Acute Rabbit Dermal Toxicity
Dow Chemical Co. HET M-4258-1 Dec. 20, 1976, Acc. 071094

A. Procedure

The undiluted test material (45.3%) was applied to the clipped unabraded abdominal area of two male and two female New Zealand Albino rabbits weighing approximately 3.0 kg. The formulation (13-14 ml) was applied at 5000 mg/kg under a heavy guage plastic-cuff, covered with a cloth bandage and secured to the marginal hair with tape. The animals were housed individually with free access to food and water. After 24 hours the cuff was removed, the skin washed with soap and water, rinsed and dried. The animals were observed for signs of toxicity during exposure and for the 14 day observation period. Body weights were recorded before and after the 24 hour exposure and at 1 and 2 weeks.

B. Results

1. Rabbit dermal, male and female LD_{50}>5000 mg/kg

2. Pharmacotoxic signs - No apparent signs of toxicity.

3. Skin reaction - Slight to moderate redness, swelling, and necrosis.

4. Necropsy: Not reported.

C. Conclusion.

1. Classification of Data - from supplemental to minimum.

a. Deficiency

i. Number of animals per sex.

b. Evaluation - It is doubtful, in the opinion of this reviewer, if additional animals of either sex would have affected the LD_{50} value reported.

2. Toxicity category IV

Eye Irritation - Rabbit
Dow Chemical Co. HET M-4258-1 December 20, 1976, Acc. 071094.

A. Procedure

Twenty-four hours prior to use, the eyes of six New Zealand albino rabbits were examined and found to be free of irritation or defects. The next day 0.1 ml of the (45.3%) formulation was instilled into the conjunctival sac of the right eye of each rabbit. After 30 seconds, the eye was washed for 2 minutes under a stream of tepid, flowing tap water. Then 0.1 ml of the formulation was instilled into the left eye of each rabbit in the same manner, but this eye was not washed. The eyes were examined for corneal damage, conjunctival irritation, and internal effects at 24 hour intervals for 3 days and again at 7 and 14 days after treatment. At the 48 hour and later examinations, a drop of 5% aqueous fluorescein was instilled into each eye to aid in the assessment of corneal injury. Excess stain was removed by washing with a stream of tepid, flowing tap water.

B. Results

1. Unwashed eyes

2. Washed eyes

C. Conclusions

1. Classification of Data - Supplemental

a. Deficiency

i. Method of scoring eye injury was not reported.

Dermal Irritation - Rabbit
Dow Chemical Co. HET M-4258-1, December 20, 1976, Acc. 071094

A. Procedure

The backs of 6 New Zealand Albino rabbits were clipped free of hair with electric clippers 24 hours prior to use. Under a surgical gauze patch held in place with adhesive tape, 0.5 ml of the (45.3%) test material was applied to an intact and an abraded site on each animal. The patches were loosely covered with a piece of heavy gauge plastic to retard evaporation. Plastic collars were placed on the rabbits to keep them from licking the material. After 24 hours, the patches were removed and each site was assessed; the severity of the reaction was recorded then, and at 72 hours from the beginning of the test. The experimental procedure and method of rating are those for compliance with the Federal Hazardous Substances Act.

B. Results

Slight irritation and edema of both intact and abraded areas, were reported at 24 hours for 4/6 rabbits that persisted for 72 hours with a score of 1.5/8.0.

C. Conclusion

1. Classification of Data Core Guideline

2. Toxicity Category IV

2. Petition OF2423 and OH5277

In the review of March 25, 1982 by Landolt the following recommendation were made to be contingent to establishing these tolerances.

a. A subchronic neurotoxicity study (Refer to memo of 11/12/81 from Landolt to Ellenburger).
The acute delayed neurotoxicity study showed

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chlorpyrifos-methyl to be positive and/or equivocal histopathological evidence of neurotoxicity. In order to determine the neurotoxic no effect level from repeated exposure a subchronic neurotoxicity study is required. The proposed protocol for the subchronic neurotoxicity study should be approved by EPA prior to initiating the study.

i. Response from Dow Chemical with letter of September 14, 1982.

"In response to Item 1, additional details of the neurological phase of the following subchronic mammalian toxicological study are attached, Appendix B.

Letter to R. D. Vatne from R. J. Kociba

Monkey Neurotoxicity Study, Pesticide Petition OF2423, Section C.2.19.0

As discussed with EPA personnel in a conference on March 16, 1982, the aforementioned study is submitted in accordance with 40 CFR § 163.82-5(b) in lieu of the requested subchronic neurotoxicity study in the chicken."

In the letter to R. D. Vatne from R. J. Kociba, the following conclusions were reported in Appendix B.

"Thus, the original histopathologic examination of monkeys used in the 6-month study of DOWCO 214 included a thorough examination of major tissues from the peripheral and central nervous systems with no indications of morphologic lesions of the nervous system induced by treatment with DOWCO 214. My evaluation leads me to concur with those conclusions."

ii. Reviewers comment

We did not agree to accepting the six month monkey feeding study in lieu of a subchronic neurotoxicity study without first reevaluating the merits of the monkey feeding study. The re-examination of the histopathology of the six month monkey feeding study is compatiable with the positive findings reported for the acute chicken delayed neurotoxicity study. However, the six month monkey feeding study was inconclusive for evidence of systemic or cholinesterase

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affects at the dosage levels tested. This study is deficient for the lack of mean values and appropriate statistical analysis reported for body weights, hematology, clinical chemistry or plasma and erythrocyte cholinesterase activities for each dosage level at the interval sampled. Considering the deficiencies in the six month monkey feeding study observed by this reviewer and in the secondary review by T. Edwards for its relevance to a subchronic neurotoxicity study, reference is made to the original request for a subchronic neurotoxicity study on this chemical.

b. Mutagenic study - refer to memo of 12-21-81 from Mauer to Landolt.

These assays appear to have been conducted with appropriate control procedures, adequate to generate valid data. The report, however, lacks certain explicit information (not supplied in the sections on experimental protocol, results and conclusions, or evaluation criteria), the inclusion of which would have substantiated the interpretation of the results. The following deficiencies in the report are noted:

1. The purity of the test compound as received, and its solubilities (especially in DMSO).

2. The number of plates per treatment. Or, do the values (revertents or convertents per plate) represent single counts from single plates?

3. The number of replicate assay performed. (It is customary do duplicate a test at least once.)

4. The genetic characteristics of the strains, and the assurance of their integrity throughout the test.

5. The type of genetic change being detected (tested for) in each tester organism. (For example, reversion to histidine-competency in Salmonella; presumably, gene conversion at the typtophan locus in a heterozygous diploid strain of yeast.)

6. The qualitative notation about cytotoxicity: "...the high dose exhibited some physiological effect and the low dose was below the toxic level..." is insufficient for the assurance the appropriate range of concentrations of the test compound spanning 10% to 90% survival were tested.

7. No statistical analyses are presented.

In view of these shortcomings in reporting, the conclusion that the assays have demonstrated no genetic activity of DOWCO 214 in these microbial tests remains qualified (uncertain).

i. Response from Dow Chemical with letter of September 14, 1982.

"Even though the test protocols have changed, the mutagenicity studies conducted by Bionetics were run in accordance with 1975 laboratory procedures. Therefore it is felt that this test is valid. In support of this conclusion a letter from D. J. Brusick to R. D. Vatne is submitted, Appendix C, which provides additional information pertinent to the study. The purity of the sample tested is unknown. Other tests conducted at this time used chlorpyrifos-methyl with an assay of 99.2%."

In the letter from D. J. Brusick of Litton Bionetics to R. D. Vatne the following responses were received in Appendix C.

2.a. "The purity of the test compound as received, and its solubilities, expecially in DMSO."

Response: The test material was not analyzed for purity by Litton Bionetics, Inc. because it was submitted as a coded compound DOWCO 214 and methods for compound identity were not available. It was not a practice at that tme to conduct independent purity or identity studies. The client was assumed to have documentation for ese parameters.

The test material was soluble in DMSO.

2.b. "The number of plates per treatment, or whether the values (revertents or convertents per plate) represent single counts from single plates."

Response: The procedures used in the 1975-1976 period followed the procedures paper of Ames, et al. 1975. Each data point represented the plate count from a single plate per dose or control point.

2.c. "The number of replicate assays performed."

Response: See above. It was not customary to conduct an independent repeat unless the test

parameters suggested some portion of the assay was out of specifications. That was not the case in this test. Independent retest of all assays has been recommended by OECD and EPA GenTox protocols established in the 1979-1980 period.

2.d. "The genetic characteristics of the strains, and the assurance of their integrity throughout the test."

Response: Each tester strain was evaluated for the following traits before use in the assay:

1. Spontaneous reversion frequency to his+
2. Sensitivity to crystal violet
3. Sensitivity to ampicillin
4. Presence of non Salmonella contaminants

2.e. "The type of genetic change being detected (tested for) in each tester organism. (For example, reversion to histidine-competency in Salmonella; presumably, gene conversion at the tryptophan locus in a heterozygous diploid strain of yeast.)"

Response:

Strain Designation	Gene Affected	Additional Mutations			Mutation Detected
		Repair	LPS	R Factor	
S. typhimurium:					
TA-1535	his G	uvr B	rfa	-	Base-pair substitution
TA-1537	his C	uvr B	rfa	-	Frameshift
TA-1538	his D	uvr B	rfa	-	Frameshift
TA-98	his D	uvr B	rfa	pKM101	Frameshift
TA-100	his G	uvr B	rfa	pKM101	Base-pair substitution
Yeast D_4	trp 5-12, trp 5-27	-	-	-	Mitotic gene conversion

2.f. "The qualitative notation 'the high dose exhibited some physiological effect and the low dose was below the toxic level' is insufficient to ensure that the appropriate range of concentrations of the test compound spanning 10 to 90° survival were tested."

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Response: In 1975-1976 the LBI test protocol followed the procedures paper of Ames, et al, 1975. The dose range specified by Dr. Ames in this paper was "up to 500 ug per plate." This paper was considered an adequate representation of the state-of-the-art in 1975-1976. Presently, an upper dose of 10,000 ug per plate is employed.

2.g. "Statistical analyses."

Response: The evaluation criteria employed in this assay were patterned after the recommendation of Ames, et al., 1975. No statistical analysis of the results were performed. Positive effects were based on the presence of dose response effects and/or fold increases in revertant counts compared to the solvent control plates.

ii. Reviewers comment: Memo of October 13, 1982 from Dr. Mauer to Landolt.

2.a. (Purity, etc.) - Insufficient; and to what extent was "test material soluble in DM50"

2.b. (No. of plates, etc.) Insufficient, according to established criteria.

2.c. (No. of replicate assays.) Accepted.

2.d. (Genetics of strain/responses.) Accepted.

2.e. (Type of genetic changes.) Accepted

2.f. (Range of Roses for acceptable assay.) Accepted.

2.g. (Statistics, lack of.) Accepted.

Conclusion: Test should be re-run with current criteria for Ames Testing.

c. Teratology study in second species is deficient. In the rabbit teratology study the experimental method is not clear on whether the test material was administered in the diet or by oral intubation.

i. Response from Dow Chemical with letter of September 14, 1982.

"The method of administration used in report C.2.29.0 is described in section B of this report."

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From Report C.2.29.0 Section B items 6 and 7.

"Chlorpyrifos-methyl was dissolved in a small amount of acetone and mixed with corn oil. 2 ml diet/kg/day was orally administered daily by stomach catheter. The concentration of chlorpyrifos-methyl in the food was adjusted on the basis of animal weight and average daily food consumption. 2 ml corn oil/kg/day was administered to control group."

ii. Reviewers Comment:

It is not clear whether chlorpysifos-methyl was administered by stomach catheter or in the food.

e. Rat reproduction study.

A no observable effect level was not determined in the rat reproduction study (Dow Report NBX-021).

i. Response from Dow Chemical with letter of September 14, 1982 appendex D.

"Pup weights show a significant decrease only for the low dose group on day 21 in the F_3B generation and the high dose group on day 4 of the F_3B generation. The existence of two statistically significant findings among the large number of comparisons (36 comparisons for combined body weights alone plus the separate male and female analyses) is not indicative of a treatment effect particularly since the results are not repeatable across generations and do not appear to follow a consistent dose response relationship."

ii. Reviewers Comment:

This reproduction study was determined to be supplementary with the deficiencies of only two dosage levels tested and a no effect level was not determined. Dow Chemical has submitted an amendment to Table 17 of the reproduction report NBX-021 Acc. No. 242150 with a statistical analysis of the pup weights to show the lack of a dose response relationship in either dosage levels for the F 3b litters. A significant depression of plasma and erythrocyte cholinesterase activity was reported for the 1.0 and 3.0 mg/kg dosage levels in the rat reproduction study and the

2 year rat chronic feeding study. In the 2 year rat feeding study the systemic no effect level was 1.0 mg/kg and the cholinesterase no observable effect level was 0.1 mg/kg. With no observable effects apparent with the reproductive indices at 1.0 mg/kg an additional reproductive dietary level to elucidate the cholinesterase no effects at a lower level would not contribute to the intent of this reproductive study. The data classification of this reproduction study should be changed from supplementary to minimum.

Raymond E. Landolt
Review Section No. 1
Toxicology Branch/HED (TS-769)

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REVISED:DCR-46639:Little/R.Landolt:TOX:H816:X73715:efs:11/16/82

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